THE EFFECTS OF ETHYL ALCOHOL ON GROWTH AND RESPIRATION IN PELOMYXA CAROLINENSIS

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Among the first of comparatively few studies made on the effect of alcohol on unicellular organisms were those of Calkins and Lieb (1902) and Woodruff (1908). These workers used Paramecium and in general concluded that ethyl alcohol, in "moderate" concentration, acts as a stimulus in sustaining the vitality of these cells.

On the contrary, Matheny (1910) and Estabrook (1910) found no evidence that alcohol acts as a periodic or continued stimulus. In minute doses, 2% or less, it apparently has no effect on Paramecium while in doses of 3% or greater, it kills them. Daniel (1909) working with another genus, Stentor, found that ethyl alcohol causes an increase in growth rate. He also states that comparatively weak solutions of alcohol (1% or less) are ineffective, while a 4% solution causes early death.

More recently Bills (1924) observed that alcohol seems to restore starved paramecia to their former vitality. Loefer and Hall (1936) studied the effects of alcohol on several species of ciliates and flagellates. Of those studied, growth was accelerated in only two species, *Euglena gracilis* and *Euglena deses* (in 0.025 to 0.1% alcohol). Goldschmied-Hermann (1935) found that Paramecium dies in 3% ethyl alcohol. In concentrations of 1 to 2%, movements become sluggish and there is an increase in number of vacuoles and in the rhythmicity of their contractions. Three or four vacuoles were occasionally formed instead of the usual two.

Even fewer studies have been made on amoeboid cells and their reactions to alcohol. Brinley (1928) worked with Amoeba and found that 5% ethyl alcohol causes them to retract their pseudopods and take on a spherical form. The protoplasm becomes very fluid. Frederikse (1932) confirmed these results.

Daugherty (1937) investigated the effect of methyl (1.99 *M*), ethyl (1.028 *M*), propyl (0.467 *M*), butyl (0.164 *M*), and amyl (0.066 *M*) alcohols on *Amoeba proteus* and *Amoeba dubia*. In weak concentrations a liquefaction of the plasmagel occurs at first but with prolonged exposure to these concentrations the liquefied portion becomes gelated. In medium or strong concentrations of these alcohols, gelation of the plasmasol was observed.

Because of rather limited knowledge on the effects of alcohol on living cells and because of the ease with which Pelomyxa lends itself to studies of this type, the following investigations were carried out.

Materials and Methods

The organism used in these investigations, *Pelomyxa carolinensis* Wilson (also referred to as *Chaos chaos*, *Chaos carolinensis* and *Amoeba carolinensis*), was

grown in a solution developed by Pace and Belda (1944). The medium was composed of the following: K_2HPO_4 — 80 mg.; KH_2PO_4 — 80 mg.; $CaCl_2$ — 100 mg.; $Mg_3(PO_4)_2 \cdot 4H_2O$ — 2 mg.; and redistilled water to 1000 ml.

Paramecium caudatum was used as food for the pelomyxae; the latter are from 25 to 50 times as large as the former. The paramecia were grown in comparatively narrow jars (500 cc. capacity) containing the above medium plus hay infusion. A week or ten days after addition of the paramecia to a fresh culture they may be collected in abundance from the surface. Stacking dishes (finger bowls) of 10 cm. diameter, to which 150 cc. of pelomyxa solution or test solution were added, were used as culture chambers.

In ascertaining growth, both numbers of organisms and their volumes were taken into consideration. The volumes were measured by means of a volumescope (Chalkley, 1929; Belda, 1942). In these experiments the over-all average volume varies very little, except in the highest concentration of alcohol.

The rate of oxygen consumption was ascertained by means of a Barcroft-Warburg respirometer.

All the various dilutions were made from freshly prepared culture media and 100% ethyl alcohol, which was the only alcohol tested. The solutions were changed daily in an attempt to maintain the alcohol concentrations as constant as possible.

Results

1. Effect of alcohol on growth in Pelomyxa

Ethyl alcohol was diluted with the culture solution, as previously described, to the following concentrations: 0.001 *M*, 0.005 *M*, 0.01 *M*, 0.05 *M*, 0.1 *M* and 0.5 *M*. In each experiment, three culture dishes were used for each concentration and 100 ml. of solution containing 25 pelomyxae were placed in each as well as in the control cultures (no alcohol). Each culture received one ml. of washed and concentrated paramecia every second day thereafter. The stacking dishes were sealed with Lubriseal to prevent possible loss of alcohol by diffusion from the culture.

The results of four experiments are presented in Table I. It is evident that 0.5 *M* alcohol is toxic to Pelomyxa. In every experiment there was a decrease in the number of organisms placed in this concentration and in Experiment 4 they died out altogether by the eighth day. In 0.005 *M* alcohol, growth is 29% greater than in the control cultures; it is also greater in 0.01 *M* and 0.05 *M* alcohol (12% greater in both) but higher concentrations than these have a retarding effect if they do not actually kill the organisms.

2. Effect of alcohol on structure, size and activity of Pelomyxa and upon digestion of food

Other characteristics, aside from growth, are also affected by alcohol. For example, at the end of 24 hours in solutions containing 0.5 *M* alcohol, only rarely is a pelomyxa found attached to the substratum. However, in the control and in the 0.001 *M* and 0.005 *M* alcohol solutions, they seemed to be firmly attached.

The organisms are also quite active in these solutions although in 0.01 *M*, 0.005 *M*, and 0.001 *M* alcohol they are much less active and even slight jarring of the culture dish will easily dislodge them if they are attached. In spite of these differ-

TABLE I

Growth of Pelomyxa in various concentrations of ethyl alcohol

Experiment No.	Molar concentration of alcohol	No. of pelomyxae on days (as designated) following inoculation					Total increase
		2	4	6	8	10	
1	0	34	39	54	127	208	183
	0.001	32	40	52	109	173	148
	0.005	29	41	62	145	276	251
	0.01	32	38	50	131	219	194
	0.05	30	44	65	170	252	227
	0.1	26	28	32	32	34	9
	0.5	23	22	22	18	10	−15
2	0	30	34	51	107	151	126
	0.001	33	46	67	116	192	167
	0.005	36	47	71	128	203	178
	0.01	34	48	63	136	224	199
	0.05	34	45	75	141	211	186
	0.1	29	37	49	72	108	83
	0.5	26	28	26	15	5	−20
3	0	28	34	84	177	262	237
	0.001	28	35	76	183	246	221
	0.005	29	36	99	265	342	317
	0.01	29	36	77	231	321	296
	0.05	26	32	99	257	328	303
	0.1	27	29	72	138	272	247
	0.5	26	24	22	19	18	−7
4	0	35	52	74	199	271	246
	0.001	27	49	74	177	253	228
	0.005	33	41	89	208	305	280
	0.01	29	44	74	162	224	199
	0.05	30	42	68	139	204	179
	0.1	25	25	32	47	91	66
	0.5	18	10	6	0	0	−25
Average for all experiments. (32 cultures for each concentration)	0	32	40	88	152	223	198
	0.001	30	42	87	148	218	193
	0.005	32	41	80	186	281	256
	0.01	31	41	88	185	247	222
	0.05	30	41	77	177	248	223
	0.1	27	30	48	72	126	101
	0.5	23	21	19	13	8	−17

Eight cultures were used for each concentration in each experiment; 25 pelomyxae were added to each culture; counts were made every two days; temperature, 25° ± 1° C.

ences between the experimental and control animals growth is greater in 0.01 *M* and 0.05 *M* alcohol solutions.

Pelomyxae exposed to high concentrations of alcohol, especially 0.5 *M*, are very inactive and appear to have a narrow solated area. The pseudopods are short and rounded. Very few food vacuoles are found, although numerous dead paramecia

are scattered over the bottom of the dish. In Figure 1, a typical normal pelomyxa is compared with one grown in 0.5 *M* alcohol solution.

One of the most striking structural changes is found in the hyaline layer, which in normal organisms is a narrow clear area (except at the tips of pseudopods, where it is quite pronounced) found immediately beneath the plasmalemma. In pelomyxae exposed to 0.5 *M* alcohol for 24 hours, the hyaline layer becomes greatly enlarged and well-defined in all parts of the organism. The area becomes more noticeable from day to day, and appears to be most noticeable on about the fourth or fifth day of exposure. The increase in depth of the hyaline layer is noticed in all concentrations from 0.05 *M* and higher. Another noticeable change is in the decrease in size of the pelomyxae in the high alcohol concentrations. After 8 days in 0.5 *M* alcohol, they are about 1/10 their original size. This is illustrated well in the figure.

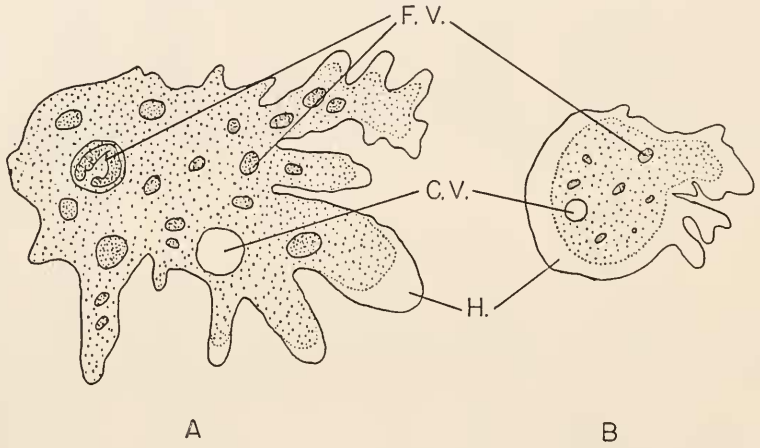

FIGURE 1. Camera lucida sketches of (A) a typical pelomyxa from a well-balanced culture solution without alcohol, and (B) one from the same solution containing alcohol in 0.5 *M* concentration. F. V., food vacuole; C. V., contractile vacuole; H., hyaline area.

The rate of digestion of food organisms is also retarded in high concentrations of alcohol. In the controls and in the 0.001 *M* to 0.01 *M* alcohol solutions, most of the food vacuoles contain only fragments of paramecia after 24 hours while in higher concentrations rather large fragments are found even after 5 days. In the 0.5 *M* alcohol solution the food vacuoles are carried over from the time of ingestion before exposure to alcohol; no paramecia are ingested by the pelomyxae after the latter are exposed to this concentration.

3. Effect of alcohol on respiration in Pelomyxa

The Barcroft-Warburg respirometer was used to ascertain oxygen consumption. This was done by means of the "direct method" as described by Pace and Belda (1944). Tests were made on pelomyxae in solutions without alcohol, and in solutions containing 0.005 *M* and 0.01 *M* alcohol. For each concentration, two sets of tests were run: in one, the organisms were introduced into the alcohol solution just

preceding the test; in the other, they were placed in the solutions 24 hours preceding the test. All the organisms were "well-fed" as described by Pace and Belda (1944).

To each Warburg flask, 500 pelomyxae were added. These were approximately uniform in size. The volumes of 30 pelomyxae selected at random were ascertained by means of a volumescope (Pace and Belda, 1944) before and after the tests and found to approximate 35,000 mm.^{3} per million organisms. The results are presented in Table II. The duration of each experiment varied between 5 and 9 hours. The oxygen consumption was ascertained in mm.^{3} per hour per million organisms as well as in mm.^{3} per hour per mm.^{3} protoplasm.

TABLE II

Oxygen consumption in Pelomyxa carolinensis in different concentrations of alcohol as compared to that in the absence of alcohol

Concentration of alcohol	Number of tests	Duration of experiment	Average rate of O^2 consumption in mm.^{3} per hr. per 10^6	Average O_2 consumption in mm.^{3} per hour per mm.^{3} cell substance
Organisms placed in test solutions just preceding test				
0 (control)	9	5–6 hrs.	9,800	0.280
0.005 M	9	5–6 hrs.	16,300	0.465
0 (control)	9	5–9 hrs.	7,200	0.205
0.01 *M*	9	5–9 hrs.	14,800	0.422
Organisms placed in solutions 24 hours preceding test				
0 (control)	9	5–7 hrs.	10,700	0.305
0.005 *M*	9	5–7 hrs.	23,500	0.671
0 (control)	9	5–7 hrs.	5,000	0.143
0.01 *M*	9	5–7 hrs.	20,900	0.568

Temperature, 25° ± 1° C.; average volume of 1 million pelomyxae, about 35,000 mm^3.

In both test solutions, the oxygen consumption is greater than in the control solutions which had no alcohol present. These results are therefore of a similar nature to those obtained in the growth studies in which growth increases appreciably in both these concentrations. In fact, the accelerating effect of alcohol is much more pronounced in the respiratory studies. The effect is even greater if the organisms are placed in the alcohol solutions for 24 hours before the tests are run.

DISCUSSION

Studies made upon ethyl alcohol and its effects on unicellular animals are not very extensive. In the earlier work cited previously, most of the tests were carried out with Paramecium as experimental animal although some observations were made upon amoeboid organisms. A comparison of the results seems to indicate that the amoeboid cells react to alcohol in a manner somewhat similar to paramecia which were usually killed by concentrations around 3% (0.75 *M*).

In the tests reported here, in which observations were made at various concentrations, most of the pelomyxae that had been put into 0.5 *M* (2.3%) alcohol solutions were dead at the end of 10 days; on the average, only 8 out of every 25 organisms were still alive.

In lower concentrations (0.005 *M* and 0.01 *M* alcohol) there was an actual increase in growth of these organisms. Associated with this growth increase is a very noticeable acceleration in oxygen consumption of the pelomyxae in the same concentrations of alcohol. This would suggest that the energy released on the oxidation of alcohol is available for certain processes in Pelomyxa.

Evidently much of the energy produced by this means is wasted; at least this is true if growth can be used as a measure for assimilative metabolism. For example, the optimum alcohol concentration for growth was found to be 0.005 *M*, in which there were produced on the average 281 organisms from 25 pelomyxae over a period of 10 days. This is a total increase of 256 organisms, and represents an increase of 30% over the control organisms without alcohol. On the other hand, in this same concentration of alcohol, there was a much higher percentage increase in oxygen consumption; a 66% increase in the organisms that were not adjusted and 119% in those that had lived in the alcohol for 24 hours before oxygen consumption was ascertained.

Summary

1. Specimens of *Pelomyxa carolinensis* were exposed to various concentrations of ethyl alcohol and observations made upon their rate of growth and respiration. The following concentrations were tested: 0, 0.001 *M*, 0.005 *M*, 0.01 *M*, 0.05 *M*, 0.1 *M*, and 0.5 *M* and 1.0 *M*.

2. In 1.0 *M* (4.6%) alcohol all the organisms were dead within 24 hours. In 0.5 *M* (2.3%), although most of them died early, some lived for a 10-day period.

3. Growth was accelerated in 0.005 *M*, 0.01 *M*, and 0.05 *M* alcohol; the greatest acceleration was a 30% increase over the control in 0.005 *M*.

4. In the higher concentrations of alcohol (0.5 *M* and 0.1 *M*) the pelomyxae do not feed and show considerable decrease in size; the hyaline layer becomes very large.

5. Rate of respiration was found to be much greater in 0.01 *M* and 0.005 *M* alcohol than in the controls without alcohol. It was greatest in 0.01 *M*, especially when the organisms were put into the alcohol solution for 24 hours before the tests were run, in which case respiration was 318% greater than in the controls.

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